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Chromosome 22q11.2 Microdeletions in Velocardiofacial Syndrome Patients With Widely Variable Manifestations

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Velocardiofacial syndrome (VCFS) and the DiGeorge sequence (DGS) are caused by 22q11.2 deletions. Fluorescence in situ hybridization (FISH) using the DiGeorge chromosome region (DGCR) probe (Oncor) was used to detect 31 deletions in 100 patients with possible VCFS. Retrospective FISH analysis of archived slides from 14 patients originally studied only by high-resolution G banding detected 6 patients with a DGCR deletion, and only 2 of these 6 had a microscopically visible chromosome deletion. The 4 familial deletions found exhibited a wide range of clinical presentations within each family. Comparison of clinical characteristics of patients with and without the DGCR deletion determined findings predictive of the deletion: abundant or unruly scalp hair; narrow palpebral fissures; a laterally "built-up" nose; velopharyngeal inadequacy; thymic hypoplasia; and congenital heart defects, specifically tetralogy of Fallot, ventriculoseptal defect, and interrupted aortic arch. © 1996 Wiley-Liss, Inc.

KEY WORDS: velocardiofacial syndrome, DiGeorge sequence, deletion 22q11, fluorescence in situ hybridization

INTRODUCTION

Velocardiofacial syndrome (VCFS) [Shprintzen et al., 1978] is an autosomal dominant disorder characterized by clefts of the secondary palate, ventriculoseptal defects, characteristic facial findings, and developmental delay. The DiGeorge sequence (DGS) can occur as a component of velocardiofacial syndrome [Carey, 1980; Lammer and Opitz, 1986]. DGS is a developmental field defect of the third and fourth pharyngeal pouches, which results in thymus and parathyroid gland hypoplasia and conotruncal heart defects. Autosomal dominantly inherited cases of DGS may represent examples of velocardiofacial syndrome [Stevens et al., 1990].

Cytogenetic analysis of DGS patients demonstrated a variety of abnormalities involving chromosome 22, including loss of 22pter–22q11 [Back et al., 1980; de la Chapelle et al., 1981; Greenberg et al., 1984; Bowen et al., 1986; Faed et al., 1987], interstitial deletion within 22q11 [Greenberg et al., 1984; Mascarello et al., 1989], and an apparently balanced translocation disrupting 22q11 [Augusseau et al., 1986]. Molecular genetic analysis of DNA markers from 22q11 demonstrated the presence of submicroscopic deletions in DGS patients, without visible cytogenetic abnormalities [Scambler et al., 1991; Driscoll et al., 1992; Desmaze et al., 1993]. Subsequently, molecular studies of velocardiofacial-syndrome patients demonstrated deletion of DNA probes from the same region as DGS, suggesting that these disorders may represent part of a spectrum of abnormalities associated with 22q11 microdeletion [Driscoll et al., 1992; Scambler et al., 1992; Wilson et al., 1992, 1993; Kelly et al., 1993].

The introduction of fluorescence in situ hybridization (FISH) into the clinical cytogenetics laboratory has greatly facilitated the characterization of chromosomal abnormalities [reviewed in Trask, 1991; Sawyer et al., 1992; Cohen et al., 1993]. FISH to metaphase chromosomes is an efficient method of detecting microdeletions associated with many diseases, including VCFS and DGS [Kuwano et al., 1991; Desmaze et al., 1992, 1993; Lebo et al., 1993; Fisher and Scambler, 1994].

This study presents a series of patients suspected of having VCFS, with or without DGS, who were referred for cytogenetic testing. The objectives of this study were to (1) confirm the VCFS diagnosis, (2) extend the description of the disease phenotype, and (3) study the inheritance of the deletion.

MATERIALS AND METHODS

Clinical Findings

Patients suspected of having velocardiofacial syndrome with or without DGS were referred to the UCSF Cytogenetics Laboratory from the Center for Craniofa-

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cial Anomalies, Genetics Clinic, Intensive Care Nursery, or Pediatric Ward. Most patients referred for this study were examined by a clinical geneticist (M.G.) and had at least two of the following characteristics: facial anomalies characteristic of VCFS, frequent ear infections, hearing loss, palate/velopharyngeal abnormalities, thymic hypoplasia, hypocalcemia, congenital heart defect, hypotonia, developmental delay, or language delay. Complete clinical information was obtained from the patients' medical charts, most of which included a consult report by Craniofacial or Genetics Clinic physicians. Clinical findings typical of VCFS noted in the patients' charts were recorded and tabulated separately according to the DiGeorge chromosome region (DGCR) deletion status.

Cytogenetics

Laboratory studies of possible VCFS patients included complete cytogenetic analysis and preparation of two GTG-banded karyotypes and FISH to detect the chromosome 22 deletion associated with VCFS and DGS. The DGCR probe mix (Oncor, Inc., Gaithersburg, MD) was used according to the manufacturer's directions. This mix contains DGCR probe N25, which hybridizes to locus D22S75 at 22q11.2, and a control probe, which hybridizes to locus D22S39 at 22q13.3.

Briefly, unheated slides were incubated in 2× SSC (20×SSC stock: 300mM NaCl plus 30mM Na citrate) at 37°C for 30 min, denatured in 70% formamide plus 2× SSC at 72°C for 2 min, and hybridized to digoxigenin-labeled probe overnight at 37°C. After hybridization, slides were washed in 50% formamide plus 2× SSC at 43°C for 15 min and then in 2× SSC at 37°C for 8 min. The digoxigenin-labeled probe was detected with FITC (fluorescein-5-isothiocyanate)-labeled anti-digoxigenin (Boehringer Mannheim, Inc., Indianapolis, IN), and the chromosomes were stained with propidium iodide.

Slides that had been archived for up to 1 year from patients with possible VCFS and DGS referred to the UCSF Cytogenetics Laboratory prior to the availability of the DGCR probe were analyzed by FISH. The hybridization protocol for archived slides followed Lebo et al. [1993].

FISH results were analyzed by examining the fluorescent signal on metaphase spreads. A metaphase spread was scored only if the control probe hybridized to the distal region of both chromosome 22s and the DGCR probe hybridized to the 22q11.2 region of one chromosome 22. The cell was then scored for the presence or absence of DGCR hybridization to the other homolog. A minimum of 10 metaphase spreads was scored per sample; a patient was classified as not having a deletion when at least 9 of 10 cells appeared normal. A patient was considered to have a deletion when all metaphase cells scored appeared deleted.

Cytogenetic testing was offered to parents and siblings of all VCFS patients with a DGCR deletion. Laboratory analysis of these individuals included analysis of 5 Giemsa-banded metaphase cells, 1 karyotype, and FISH with the DGCR probe. FISH analysis included an examination of at least 20 metaphase spreads, thereby ruling out 14% of mosaicism with 95% confidence when all cells scored showed intact hybridizing regions.

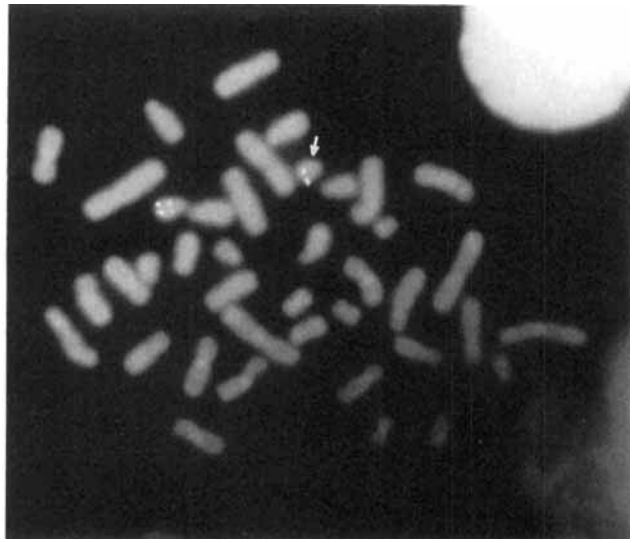

Fig. 1. FISH of the DGCR probe to a VCFS patient with a deletion in chromosome band 22q11.2. The chromosomal DNA is stained with propidium iodide and fluoresces red (gray); the DGCR probe is labeled with fluorescein and fluoresces yellow (white dots). The control probe hybridizes to the D22S39 locus at 22q13.3 on both chromosome 22s; the DGCR probe hybridizes to D22S75 at 22q11.2 on only one chromosome 22 (indicated by arrow), thus establishing the presence of a deletion of this chromosomal region on one homolog.

RESULTS

Cytogenetic Results

This study was conducted in two phases: a retrospective study of patients referred to the cytogenetics laboratory before the DGCR FISH probe became available, and a prospective study of cases referred after the test became available in our clinical laboratory. Of the 86 prospective patients with possible VCFS referred for FISH testing, 25 had a detectable deletion of the DGCR in 22q11.2 (Fig. 1). Archived slides were used for the retrospective study of 14 patients with suspected VCFS who had originally been studied by only high-resolution G banding. Two of the 14 individuals had cytogenetically visible del(22)(q11.21q11.23) confirmed by FISH. One patient previously diagnosed with del(22)(q11.21q11.23) by high-resolution G banding did not have a deletion of the DGCR detectable by FISH. Four more retrospective cases with normal G-banded karyotypes were found to have a submicroscopic deletion encompassing the DGCR locus. Altogether, 31 patients with 22q11 microdeletions were identified in 100 patients referred for VCFS.

In addition to FISH analysis for the DGCR deletion, all referred patient samples were studied by traditional cytogenetic analysis including chromosome counts of 20 cells, analysis of 5 cells, and preparation of 2 GTG-banded karyotypes. Four patients without DGCR deletions had abnormal karyotypes not involving chromosome 22: a 2-month-old boy with 46,XY,dup(4)(q31.3q33?)de novo, a 3-month-old girl with 46,XX,del(8)(p23.1) de novo, a 3-year-old boy with a karyotype of 46,XY,-1,+der(1)t(1;D or G)(p36;p12), and a 6-year-old girl with a complex karyotype of 45,X/46,X,i(Xq)/47,X,i(Xq),i(Xq).

Clinical Findings

Clinical histories of patients were tabulated and analyzed separately according to DGCR deletion status.

TABLE I. Clinical Findings of Patients With a Deletion of the DGCR Detected by FISH[a]

Age at referral	1d	1d	2d	3d	4d	3w	4w	4w	7w	4m	5m	9m	1y	2y	2y	2y	2y	2y	3y	3y	4y	4y	4y	5y	10y	10y	13y	23y	28y	34y	37y
Sex	M	F	F	F	F	M	M	M	M	F	F	F	M	F	F	F	M	M	F	M	M	F	M	M	F	M	M	F	F	M	F
Ethnicity	Af	E	H	Af	H	E	E	E/N	E	E	E/H	E/Af	E	E	E	H	E	E	Af	E/H	E	H	E	E	E	Af	As	E	A	E	H
Family	A										C	C						D		C		B		D				C	A	D	B
Growth and development																															
Small stature/growth delay						+			+		+	+	+	+	+	+			+		+	+				+		+			+
Developmental delay			+									+					+	+	+		+		+	+		+		+	+		+
Language delay/apraxia											+					+	+	+		+	+	+	+	+	+	+		+		+	+
Craniofacial anomalies																															
Microcephaly		+	+							+	+			+							+	+									
Narrow bitemporal diameter	+	+	+	+		+																		+		+				+	
Narrow palpebral fissures		+	+		+		+					+		+			+	+			+	+					+			+	
Minor ear anomalies	+	+		+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+		+	+		+		+	+	+
Laterally built up nose	+	+	+	+		+	+	+		+		+			+		+	+	+		+	+		+	+		+	+	+		+
Prominent tip of nose		+	+		+	+	+		+	+	+			+				+	+	+		+		+				+	+	+	+
Small nasal alae		+	+	+	+	+		+	+		+						+	+		+		+		+	+		+			+	+
Small, downturned mouth		+	+	+	+				+	+	+	+		+				+				+		+	+						+
Micrognathia/retrognathia		+	+			+			+		+						+				+	+						+			
Velopharyngeal inadequacy											+				+				+	+	+				+	+		+			
Cleft palate												+	+												+		+					+
Cardiac defects																															
Tetralogy of Fallot		+							+										+								+				
Atrial septal defect			+	+	+		+		+																	+						
Ventriculoseptal defect	+	+	+	+	+		+	+	+	+				+		+			+					+		+	+		+		
Patent ductus arteriosus	+	+		+	+			+	+																		+				
Pulmonic stenosis/atresia		+					+		+	+									+								+				
Truncus arteriosis															+																
Interrupted aortic arch			+	+	+	+		+						+																	
Right aortic arch																											+				
Aortic stenosis/atresia	+		+																												
Other																															
History of hypotonia		+	+				+							+		+	+	+					+	+						+	
Abundant/unruly scalp hair			+								+			+								+									+
Frequent ear infections											+	+	+				+			+	+		+		+	+	+	+		+	
Hearing loss																	+									+					
Thymic hypoplasia				+		+			+					+	+		+														
Hypocalcemia				+	+				+									+					+								
Long, slender fingers		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+		+	+			+	+		+
Unusual findings													a					b						c	d						

[a]Sex: M = male, F = female. Ethnicity: As = Asian, Af = African American, E = European, H = Hispanic, N = Native American. Unusual findings: a = cleft lip, b = severe lordosis, c = craniosynostosis, hypospadias, club feet, d = spider hemangiomas.

Clinical findings of the 31 patients with DGCR deletions are listed according to age in Table I. Both sexes were represented equally, with 16 female and 15 male patients. These American patients had European, African, Asian, Hispanic, and Native American ancestors. The most common manifestations, present in over half of the patients, included minor ear anomalies; a laterally built-up nose with prominent tip and small nasal alae; long slender fingers; and congenital heart defect, specifically ventriculoseptal defects. The clinical presentation of patients differed by age of diagnosis. Neonatally, heart defects, typical face, and long fingers were found in almost all of the deletion patients. Older patients had small stature or growth delay, developmental and language delays, and velopharyngeal problems, usually without heart defects. Unusual findings in individual DGCR-deletion patients included cleft lip, severe lordosis, craniosynostosis, hypospadias, club feet, and spider hemangiomas.

A comparison of the clinical findings of patients with and without DGCR deletions was undertaken to find manifestations that might predict the deletion (Table II). Anomalies found more often in patients with the DGCR deletion than in those without included abundant or unruly scalp hair; narrow palpebral fissures; a laterally built-up nose; velopharyngeal inadequacy; thymic hypoplasia; and congenital heart defects, specifically tetralogy of Fallot, ventriculoseptal defect, and interrupted aortic arch. All patients with DGS as a component of VCFS were found to have the DGCR deletion.

Inheritance

Parents of all deletion patients were offered cytogenetic testing to study deletion transmission and clarify familial recurrence risks. Ten sets of both parents and 8 individual parents (1 father and 7 mothers) were tested for the DGCR deletion. The remaining parents declined or were unavailable for testing. Four familial DGCR deletions were found: 3 deletions were inherited from clinically affected mothers whose partners were not available for testing and 1 deletion from a clinically affected father. One other mother clinically affected with VCFS declined cytogenetic testing. No evidence of deletion or mosaicism for the deletion was found in clinically normal parents who were tested.

A wide range of clinical presentations was observed in families with more than 1 affected relative. The variability in disease presentation in these families may reflect variability in the course of the disease, or it may be a reflection of the age at presentation to the clinic. Fam-

TABLE II. Comparison of Clinical Findings in Patients Positive and Negative for DGCR Deletion

FISH results	% Positive (n = 31)	% Negative (n = 47)
Average age at referral	5.2 years old	3.7 years old
Growth and development		
Small stature/growth delay	45	36
Developmental delay	39	51
Language delay/apraxia	45	45
Craniofacial anomalies		
Microcephaly	23	36
Narrow bitemporal diameter	26	23
Narrow palpebral fissures	35	13
Minor ear anomalies	77	55
Laterally built-up nose	65	28
Prominent tip of nose	55	32
Small nasal alae	55	28
Small downturned mouth	45	26
Micrognathia/retrognathia	29	34
Velopharyngeal inadequacy	26	4
Cleft lip	3	11
Cleft palate	16	19
Cardiac anomalies		
Tetralogy of Fallot	13	6
Atrial septal defect	19	9
Ventriculoseptal defect	52	13
Patent ductus arteriosus	23	21
Pulmonic stenosis/atresia	19	17
Truncus arteriosis	3	4
Interrupted aortic arch	19	2
Right aortic arch	3	2
Aortic stenosis/atresia	6	9
Other		
History of hypotonia	29	19
Abundant or unruly scalp hair	16	17
Frequent ear infections	39	26
Hearing loss	6	17
Thymic hypoplasia	19	0
Hypocalcemia	16	11
Long slender fingers	74	53

ily A consisted of a 1-day-old baby boy and his 28-year-old mother. The mother was mentally retarded and had a ventriculoseptal defect and typical facial anomalies. Her son had complex congenital heart disease that included a ventriculoseptal defect, patent ductus arteriosus, and aortic atresia. He had the typical newborn face and died a few weeks after birth. Neither had any noted velopharyngeal problems.

Family B included a 4-year-old girl and her 37-year-old mother. Both mother and daughter had small stature, language delay, and typical face. The mother also had developmental delay and a repaired cleft palate. The daughter's palate was normal.

Family C (Figs. 2, 3) was seen by physicians in the Center for Craniofacial Anomalies and the Genetics Clinic. FISH testing of 3 generations demonstrated a de novo DGCR deletion in a 23-year-old woman who transmitted it to her 3 children. The mother and 2 of her children (III-1 and III-2) had velopharyngeal inadequacy, and the youngest (III-3) had a cleft palate. Daughter III-2 had required a tracheostomy for Pierre Robin sequence in infancy. All had varying degrees of growth and developmental and language delay in addition to typical VCFS facial appearance and long fingers. The mother had significant psychiatric and developmental problems. None had detectable cardiac or thymic anomalies.

Family D included two boys, aged 2 and 5 years old, who had inherited a deleted chromosome 22 from their 34-year-old father. The younger boy had hypocalcemia in infancy, developmental and language delay, and was hypotonic. The older boy had developmental and language delay, craniosynostosis, hypospadias, club feet, and a ventriculoseptal defect. The father had a history of childhood hypotonia, language delay, and frequent ear infections. Both boys had characteristic facial findings that were more subtle in the father.

DISCUSSION

Because most deletions associated with VCFS and DGS are submicroscopic and because chromosome analysis can be ambiguous, FISH results are more accurate and easier to interpret than high-resolution Giemsa-banded karyotypes. Our retrospective studies detected 3 times as many deletions by FISH as by high-resolution cytogenetic analysis, in agreement with other studies that detected up to 5 times as many deletions by FISH [Scambler et al., 1991; Desmaze et al., 1993]. Although in situ hybridization confirmed 2 cases previously reported to have a visible deletion of 22q11, our inability to confirm a third reported deletion may be due to the presence of a deletion of a nearby region not encompassing the DGCR probe locus or to an originally incorrect Giemsa-banded chromosome interpretation.

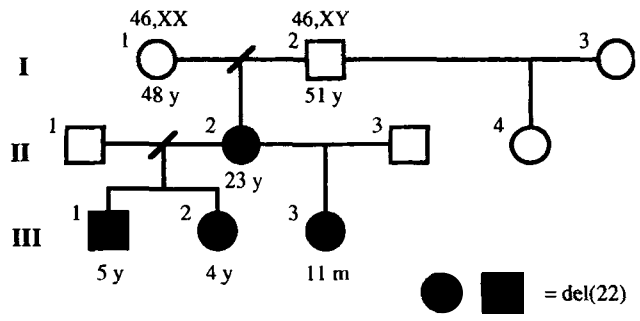

Fig. 2. Pedigree of Family C. Filled symbols represent patients with a DGCR deletion. I-1 and I-2 had normal karyotypes with no DGCR deletion detected by FISH.

This clinical study was undertaken with loose inclusion criteria to define the VCFS phenotype better, to identify mildly affected patients, or to exclude VCFS in patients with a broad differential diagnosis. The reported frequencies of 22q11.2 deletions detected by molecular methods range from 70% to 100% in VCFS patients [Scambler et al., 1992; Driscoll et al., 1993; Kelly et al., 1993] and from 80% to 100% in DGS patients [Carey et al., 1992; Driscoll et al., 1992, 1993]. This study found DGCR deletions in about 30% of tested patients, with a wide spectrum of VCFS findings.

Traditional cytogenetic analysis found chromosome abnormalities not known to be associated with VCFS in 4% of patients referred for this study. This is very similar to frequencies reported in routine testing for the fragile X syndrome, in which 3–5% of referred patients have unrelated chromosomal abnormalities [Wang et al., 1993; Howard-Peebles, 1994]. These findings highlight the importance of performing traditional cytogenetic analysis and FISH for suspected VCFS. Several of the suspected VCFS patients with normal karyotypes and without a detectable DGCR deletion have been given other diagnoses: infant of diabetic mother, fetal alcohol syndrome, Stickler syndrome, CHARGE association, and Marfan syndrome. A few of the non-deletion patients are still believed to have VCFS on clinical grounds. These patients may have a deletion of an adjacent region of 22q11 not detected by the DGCR probe or a small deletion or point mutation in a critical gene not detected by FISH.

Inheritance of the 22q11.2 deletion from a mildly affected parent has been reported in 8% of DGS and VCFS cases [Driscoll et al., 1993]. Most reported inherited cases of VCFS are maternally transmitted [Driscoll et al., 1992; Kelly et al., 1993; McLean et al., 1993]. Our finding of maternal deletions in 3 of 4 familial cases of VCFS is consistent with these reports. Increased maternal transmission suggests that genomic imprinting is important in the expression of the disease phenotype. However, parent-of-origin studies [Driscoll et al., 1992] and inheritance of unbalanced translocations [de la Chapelle et al., 1981; Greenberg et al., 1984; Augusseau et al., 1986] have demonstrated deletion of both maternal and paternal chromosome 22 in DGS, suggesting that genomic imprinting is not important in this disease. To our knowledge, paternal uniparental disomy of chromosome 22 has not been documented, but maternal uniparental disomy 22 has been described in apparently normal individuals [Kirkels et al., 1980; Palmer et al., 1980; Schinzel et al., 1994]. More likely explanations for the preponderance of maternally transmitted deletions include ascertainment bias secondary to sociological factors or decreased reproductive fitness of affected males.

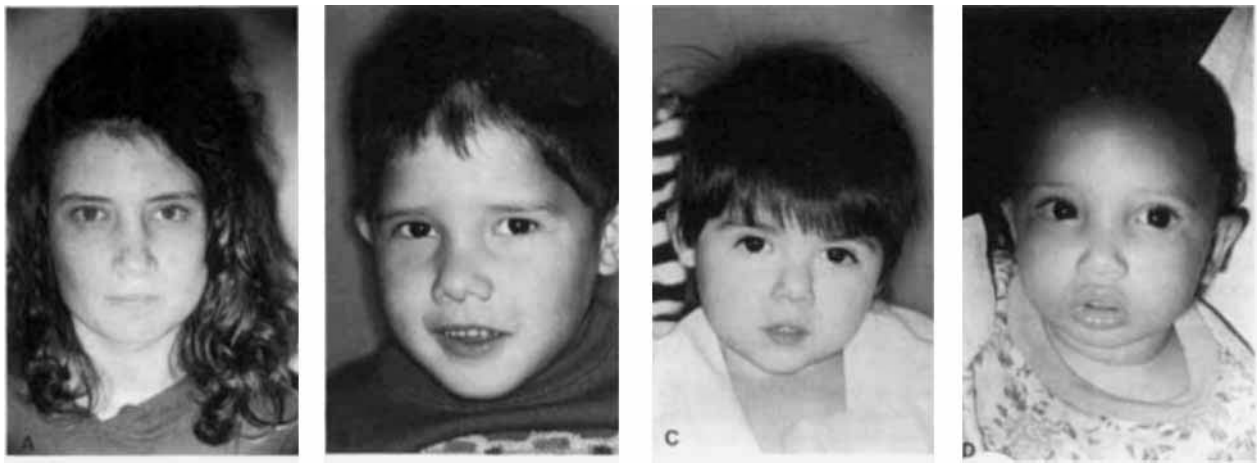

Fig. 3. Photographs of members of Family C. **A:** Individual II-2. **B–D:** III-1, III-2, and III-3, respectively.

Microdeletions are a common cause of human genetic disease [see review by Fisher and Scambler, 1994]. The complex clinical expression of many of these disorders suggests that they may be contiguous gene syndromes that result when several genes are in the deleted chromosome segment. The phenotypic variability of the disease may reflect variability of the location and extent of the deletion. However, evidence suggests that VCFS and DGS result from a single gene defect influenced by unlinked modifying genes or environment: (1) 1 DGS patient has an apparently balanced translocation t(2;22)(q14.1;q11.2) [Augusseau et al., 1986], (2) the size of 22q11.2 deletions does not correlate with DGS phenotypic severity [Scambler et al., 1991; Desmaze et al., 1993], and (3) members of DGS and VCFS families presumed to have the same deletion exhibit varying degrees of disease expression [Scambler et al., 1991; McLean et al., 1993; this report].

Because VCFS is an autosomal dominant disorder caused by a deletion, it is likely that abnormal gene dosage results in the disease. An imbalance of the DGS or VCFS gene product with respect to other gene products may disrupt gene transcription at a critical stage of embryogenesis, resulting in development of an abnormal phenotype. Characterization of mutations in VCFS patients without a large deletion will sublocalize the VCFS gene region further and ultimate identify the gene. Elucidation of this gene's product should help to explain the wide range of phenotypes observed in families with the same mutation.

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REFERENCES

Augusseau S, Jouk S, Jalbert P, Prieur M (1986): DiGeorge syndrome and 22q11 rearrangements. Hum Genet 74:206.

Back E, Stier R, Bohm N, Adlung A, Hameister H (1980): Partial monosomy 22pter→q11 in a newborn with the clinical features of trisomy 13 syndrome. Ann Genet 23:244–248.

Bowen P, Pabst H, Berry D, Collins-Nakai R, Hoo JJ (1986): Thymus deficiency in an infant with a chromosome t(18;22)(q12.2;p11.2)pat rearrangement. Clin Genet 29:174–177.

Carey AH, Clausen U, Ludecke HJ, Horsthemke B, Ellis D, Oakley H, Wilson D, Burn J, Williamson R, Scambler PJ (1992): Interstitial deletions in DiGeorge syndrome detected with microclones from 22q11. Mammal Genome 3:101–105.

Carey JC (1980): Spectrum of the DiGeorge "syndrome." J Pediatr 96:955–956.

Cohen MM, Rosenblum-Vos LS, Prabhakar G (1993): Human cytogenetics. A current overview. Am J Dis Child 147:1159–1166.

de la Chapelle A, Herva R, Koivisto M, Aula P (1981): A deletion in chromosome 22 can cause DiGeorge syndrome. Hum Genet 57:253–256.

Desmaze C, Deleuze JF, Dutrillaux AM, Thomas G, Hadchouel M, Aurias A (1992): Screening of microdeletions of chromosome 20 in patients with Alagille syndrome. J Med Genet 29:233–235.

Desmaze C, Scambler P, Prieur M, Halford S, Sidi D, Le Deist F, Aurias A (1993): Routine diagnosis of DiGeorge syndrome by fluorescent *in situ* hybridization. Hum Genet 90:663–665.

Driscoll DA, Budarf ML, Emanuel BS (1992): A genetic etiology for DiGeorge syndrome: Consistent deletions and microdeletions of 22q11. Am J Hum Genet 50:924–933.

Driscoll DA, Salvin J, Sellinger B, Budarf ML, McDonald-McGinn DM, Zackai EH, Emanuel BS (1993): Prevalence of 22q11 microdeletions in DiGeorge and velocardiofacial syndromes: Implications for genetic counselling and prenatal diagnosis. J Med Genet 30:813–817.

Faed MJW, Robertson J, Beck JS, Carter JI, Bose B, Madlom MM (1987): Features of di George syndrome in a child with 45,XX,-3,-22,+der(3)t(3;22)(p25;q11). J Med Genet 24:225–227.

Fisher E, Scambler P (1994): Human haploinsufficiency—One for sorrow, two for joy [News]. Nature Genet 7:5–7.

Greenberg F, Crowder WE, Paschall V, Colon-Linares JC, Lubianski B, Ledbetter DH (1984): Familial DiGeorge syndrome and associated partial monosomy of chromosome 22. Hum Genet 65:317–319.

Howard-Peebles PN (1994): The fragile X cytogenetic test is dead! Appl Cytogenet 20:61.

Kelly D, Goldberg R, Wilson D, Lindsay E, Carey A, Goodship J, Burn J, Cross I, Shprintzen RJ, Scambler PJ (1993): Confirmation that the velo-cardio-facial syndrome is associated with haplo-insufficiency of genes at chromosome 22q11. Am J Med Genet 45: 308–312.

Kirkels VG, Hustinx TW, Scheres JM (1980): Habitual abortion and translocation (22q;22q): unexpected transmission from a mother to her phenotypically normal daughter. Clin Genet 18:456–461.

Kuwano A, Ledbetter SA, Dobyns WB, Emanuel BS, Ledbetter DH (1991): Detection of deletions and cryptic translocations in Miller–Dieker syndrome by *in situ* hybridization. Am J Hum Genet 49:707–714.

Lammer EJ, Opitz JM (1986): The DiGeorge anomaly as a developmental field defect. Am J Med Genet Suppl 2:113–127.

Lebo RV, Lynch ED, Golbus MS, Flandermeyer RR, Yen PH, Shapiro LJ (1993): Prenatal *in situ* hybridization test for deleted steroid sulfatase gene. Am J Med Genet 46:652–658.

Mascarello JT, Bastian JF, Jones MC (1989): Interstitial deletion of chromosome 22 in a patient with the DiGeorge malformation sequence. Am J Med Genet 32:112–114.

McLean SD, Saal HM, Spinner NB, Emanuel BS, Driscoll DA (1993): Velo-cardio-facial syndrome. Intrafamilial variability of the phenotype. Am J Dis Child 147:1212–1216.

Palmer CG, Schwartz S, Hodes ME (1980): Transmission of a balanced homologous t(22q;22q) translocation from mother to normal daughter. Clin Genet 17:418–422.

Sawyer JR, Johnson MP, Miller OJ (1992): Traditional and molecular cytogenetics. J Reprod Med 37:485–498.

Scambler PJ, Carey AH, Wyse RK, Roach S, Dumanski JP, Nordenskjold M, Williamson R (1991): Microdeletions within 22q11 associated with sporadic and familial DiGeorge syndrome. Genomics 10:201–206.

Scambler PJ, Kelly D, Lindsay E, Williamson R, Goldberg R, Shprintzen R, Wilson DI, Goodship JA, Cross IE, Burn J (1992): Velo-cardio-facial syndrome associated with chromosome 22 deletions encompassing the DiGeorge locus. Lancet 339:1138–1139.

Schinzel AA, Basaran S, Bernasconi F, Karaman B, Yüksel-Apak M, Robinson WP (1994): Maternal uniparental disomy 22 has no impact on the phenotype. Am J Hum Genet 54:21–24.

Shprintzen RJ, Goldberg RB, Lewin ML, Sidoti EJ, Berkman MD, Argamaso RV, Young D (1978): A new syndrome involving cleft palate, cardiac anomalies, typical facies, and learning disabilities: Velo-cardio-facial syndrome. Cleft Palate J 15:56–62.

Stevens CA, Carey JC, Shigeoka AO (1990): Di George anomaly and velocardiofacial syndrome. Pediatrics 85:526–530.

Trask BJ (1991): Fluorescence *in situ* hybridization: Applications in cytogenetics and gene mapping. Trends Genet 7:149–154.

Wang Q, Green E, Barnicoat A, Garrett D, Mullarkey M, Bobrow M, Mathew CG (1993): Cytogenetic versus DNA diagnosis in routine referrals for fragile X syndrome. Lancet 342:1025–1026.

Wilson DI, Goodship JA, Burn J, Cross IE, Scambler PJ (1992): Deletions within chromosome 22q11 in familial congenital heart disease. Lancet 340:573–575.

Wilson DI, Burn J, Scambler P, Goodship J (1993): DiGeorge syndrome: Part of CATCH 22. J Med Genet 30:852–856.